Physiological and biochemical basis of clinical liver function tests: a review

Hoekstra, Lisette T ; de Graaf, Wilmar ; Nibourg, Geert A A ; Heger, Michal ; Bennink, Roelof J ; Stieger, Bruno ; van Gulik, Thomas M

Abstract: OBJECTIVE: To review the literature on the most clinically relevant and novel liver function tests used for the assessment of hepatic function before liver surgery. BACKGROUND: Postoperative liver failure is the major cause of mortality and morbidity after partial liver resection and develops as a result of insufficient remnant liver function. Therefore, accurate preoperative assessment of the future remnant liver function is mandatory in the selection of candidates for safe partial liver resection. METHODS: A MEDLINE search was performed using the key words "liver function tests," "functional studies in the liver," "compromised liver," "physiological basis," and "mechanistic background," with and without Boolean operators. RESULTS: Passive liver function tests, including biochemical parameters and clinical grading systems, are not accurate enough in predicting outcome after liver surgery. Dynamic quantitative liver function tests, such as the indocyanine green test and galactose elimination capacity, are more accurate as they measure the elimination process of a substance that is cleared and/or metabolized almost exclusively by the liver. However, these tests only measure global liver function. Nuclear imaging techniques ((99m)Tc-galactosyl serum albumin scintigraphy and (99m)Tc-mebrofenin hepatobiliary scintigraphy) can measure both total and future remnant liver function and potentially identify patients at risk for postresectional liver failure. CONCLUSIONS: Because of the complexity of liver function, one single test does not represent overall liver function. In addition to computed tomography volumetry, quantitative liver function tests should be used to determine whether a safe resection can be performed. Presently, (99m)Tc-mebrofenin hepatobiliary scintigraphy seems to be the most valuable quantitative liver function test, as it can measure multiple aspects of liver function in, specifically, the future remnant liver.

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1. Introduction

Liver failure is the major cause of mortality and morbidity after partial liver resection, and develops as a result of insufficient functional remnant liver (FRL) mass.[1] Post-resectional liver function largely depends on the quantity and quality of the remnant liver, the latter being inversely proportional to underlying liver disease such as steatosis, cirrhosis, and cholestasis. Assessment of liver function is therefore crucial in the preoperative work-up of patients who require (extensive) liver resection.

CT volumetry is currently the standard method to determine whether a patient can safely undergo liver resection. When using CT volumetry, a liver resection can be safely performed when FRL volume is larger than 25-30% of total liver volume in case of normal liver parenchyma.[2,3] In patients with underlying liver disease, a margin of 40% is taken into account.[4] However, liver volume does not necessarily reflect liver function, especially in patients with a compromised liver.[5,6] Therefore, it is important to reliably assess hepatic function before liver surgery in addition to CT volumetry.

Several liver function tests have been developed in the last decade, including passive liver function tests (biochemical parameters and clinical grading systems), dynamic quantitative liver function tests (time-based uptake or metabolic capacity of infused compounds), molecular nuclear imaging techniques, and bioenergetic tests. This review will primarily focus on dynamic tests for the measurement of hepatic function before liver surgery. In addition, the application of quantitative liver function tests is discussed from the perspective of preoperative risk assessment in patients with diseased liver parenchyma.

2. Definition of liver function and clinical implications

The liver is responsible for a spectrum of functions including the uptake, metabolism, conjugation, and excretion of various endogenous and foreign substances, in which transporters play an

important role (Figure 1). The liver also provides an immunological function, as the reticuloendothelial capacity of the liver plays a role in phagocytosis, and clearance of micro-organisms and endotoxins from the portal blood.[7] The secretion of bile is an important end-point of liver function and the production of bile immediately ceases when perfusion of the liver is arrested. The complexity of liver function is best reflected by our inability to restore full liver function during liver failure, insofar as liver assist devices and bioartificial livers have not proven to fully substitute all the components of liver function yet.[8,9] In addition, there is no liver function test available that measures all components of liver function.

Whereas the definition of liver function is comprehensive, a unanimous definition of posthepatectomy liver failure is lacking in literature.[10] This makes comparison between study outcomes difficult. Recently, a definition of posthepatectomy liver failure has been proposed which allows objective comparisons in future studies.[10] Nevertheless, liver surgery starts and ends with restricted information on the functionality of the organ with insufficient objective means to gauge whether the liver is failing postoperatively. Liver failure in addition depends on whether the preoperative remnant liver function has been preserved during the surgical procedure. There are many variations in surgical techniques, and possibilities of technical errors that may lead to deterioration of remnant liver function. Consequently, none of the available tests is completely accurate in predicting postoperative function of the remnant liver.[11]

3. Passive liver function tests

3.1. Bilirubin

Plasma bilirubin concentration provides indirect information on the uptake, conjugation, and excretion function of the liver. Elevated plasma concentrations of bilirubin are specific markers for serious liver injury, and therefore liver function loss. After formation of unconjugated (‘indirect’) bilirubin, it is bound to albumin for transport to the liver. Bilirubin is taken up by hepatocytes,

where it is bound by a group of cytosolic proteins, mainly glutathione S-transferases (GST), to prevent efflux from the cell.[12] Bilirubin is conjugated to glucuronic acid ('direct bilirubin') under the catalytic activity of UDP-glucuronosyltransferase 1-1 (UGT-1A), which converts conjugated bilirubin from a highly hydrophobic molecule to a relatively hydrophilic molecule.[12] Conjugated bilirubin is then excreted into the bile across the canalicular membrane as bilirubin diglucuronide by the conjugate export pump multidrug resistance protein 2 (MRP2, ABCC2).[13,14]

The specific organic anion transporting polypeptide (OATP) transporter for bilirubin remains controversial.[15] Bilirubin is transported into the cell more effectively by OATP1B1 than by OATP1B3, which was demonstrated in uptake studies using OATP1B1- or OATP1B3-transfected *X. laevis* oocytes.[16] Contrastingly, Cui et al[14] reported that the transport of bilirubin into human embryonic kidney cells (HEK293) is facilitated by OATP1B1 but not OATP1B3[15], whereas Wang et al showed that a role for OATP1B1 in bilirubin transport is unlikely.[17] However, recent pharmacogenetic studies have linked polymorphisms in the *SLCO1B1* and *SLCO1B3* genes, which encode the OATP1B1 and 1B3 isoforms, to elevated serum levels of unconjugated and conjugated bilirubin.[18-20] Similarly, mice lacking the *Sclo1a* and *Slco1b* genes exhibit a >40-fold increase in total serum bilirubin levels (predominantly conjugated bilirubin), and a 2.5-fold increase in unconjugated bilirubin, while conjugated bilirubin in serum is undetectable in the parent strain.[21] Moreover, inactivation or disruption of *Slco1b2* in mice leads to mild hyperbilrubinemia.[22,23]

Given the relevance of the OATP1B1 and 1B3 isoforms in the uptake of unconjugated and conjugated bilirubin, any liver pathology that affects OATP expression automatically alters bilirubin kinetics. For example, cytokines released by Kupffer cells during liver inflammation (e.g., during cholestasis, steatosis/steatohepatitis) and ischemia/reperfusion[24] can influence the expression of different OATP isoforms independently[25,26], and hence skew bilirubin-related test outcomes.

Additionally, bilirubin levels may also be influenced by non-hepatic factors such as an increased production as results of e.g., hemolysis during sepsis.[27] Hemoglobin and lactic acid dehydrogenase (LDH) are released during hemolysis, which result in an increase in indirect bilirubin and urobilinogen, a product of bilirubin reduction that becomes elevated in certain liver diseases such as hepatitis. Therefore, plasma bilirubin concentration is not a parameter of liver function per se in these instances. The plasma bilirubin concentration is often used in combination with other laboratory markers of hepatopathology (e.g., liver aminotransaminase levels, albumin levels), and/or clinical grading systems such as the Child-Pugh (see sections 4.1) and MELD (model for end-stage liver disease) scores.

3.2. Albumin and coagulation factor synthesis

Albumin and proteins involved in secondary hemostasis and fibrinolysis, including vitamin K-dependent coagulation proteins (factors II, VII, IX, X, protein C, protein S, and protein Z), as well as factor V, XIII, fibrinogen, antithrombin, α2-plasmin inhibitor, and plasminogen, are exclusively synthesized by the liver, and their plasma concentrations are therefore used as indirect indicators of liver synthesis function. Albumin, clotting factors, and coagulation parameters such as the international normalized ratio (INR) are measured by routine clinical chemistry. In liver disease there is a decrease in the synthesis of albumin and coagulation factors, resulting in an increase in prothrombin time (PT) and INR.

4. Clinical grading systems

Clinical grading systems such as the Child-Pugh, and MELD scores combine several biochemical parameters with clinical symptoms of insufficient liver function. For liver resections, the Child-Pugh score is deemed more relevant inasmuch as the MELD score is very narrow in patients undergoing liver resection.

4.1. Child-Pugh score

The Child-Pugh score, a widely used clinical scoring system, includes total plasma bilirubin level, plasma albumin level, and PT together with the presence or absence of encephalopathy and ascites. The Child-Pugh scoring system is particularly useful in selecting patients with HCC and cirrhosis for resection or transplantation. In Western clinical practice, most class Child B and class Child C patients are candidates for transplantation, leaving class Child A patients eligible for resection. In these Child A patients, the Child-Pugh score has been shown to be quite variable and may be unreliable for predicting the outcome of liver resections.[28-30]

5. Dynamic quantitative liver function tests

Dynamic quantitative liver function tests measure the elimination of a substance in time. Since the substances used for these tests are cleared and/or metabolized almost exclusively by the liver, the dynamic quantitative liver function tests constitute a more accurate measure of the specific aspects of liver function.

5.1. Indocyanine green (ICG) clearance test

The ICG clearance test was initially devised for the measurement of blood flow, and later employed for the assessment of liver function by measuring functional hepatocyte mass. It is now the most widely used quantitative liver function test in the clinical setting.[31] ICG is a tricarbocyanine dye that binds to albumin[32,33], alpha-1lipoproteins[28], and β-lipoproteins.[32] It distributes uniformly in the blood within 2-3 minutes after intravenous injection of 25 mg or 50 mg ICG (dissolved in 5 mL or 10 mL sterile water, respectively). ICG is exclusively cleared by hepatocytes via OATP1B3 and sodium-taurocholate co-transporting polypeptide (NTCP)[15], and excreted into the bile without biotransformation[32,34,35] by the ATP-dependent export pump

multidrug-resistance-associated protein 2 (MRP 2).[36] Under normal conditions, ICG has a high hepatic extraction rate, and its uptake is rate-limited by blood flow.[15,33] Additionally, the clearance of ICG from the blood is similar to that of various endogenous and exogenous substances such as bilirubin, hormones, drugs, and toxins. The ICG clearance test therefore reflects several important functional parameters of the liver, including blood flow-dependent clearance and transporter capacity.[15]

Elimination of ICG from the blood is dependent on hepatic blood flow, cellular uptake, and biliary excretion.[35,37] Following administration, the blood level falls exponentially for about 20 minutes, by which time approximately 97% of the dye is excreted into the bile.[34] ICG clearance is determined by serum sampling or pulse dye densitometry using an optical sensor placed on the finger.[38-40] The results of ICG tests can be expressed in several ways, including the plasma disappearance rate (ICG-PDR), the ICG elimination rate constant (ICG-k), and the ICG-R15, which describes the percent of clearance of ICG at 15 minutes or, conversely, circulatory retention of ICG during the first 15 minutes following bolus injection.[41] ICG-PDR is the most commonly used parameter, with normal ranges between 16-25%/min.[32,37,42]

The ICG clearance test has been widely used in critically ill patients, and in patients with chronically reduced hepatic function.[32,37] For example, it has been reported that the ICG-R15 value is a better indicator of liver function than the Child-Pugh classification in patients who had undergone cardiac surgery. In these patients a high ICG retention rate at 15 minutes correlated with a high rate of mortality.[43] Furthermore, the ICG clearance test was found to be a better discriminating preoperative test for evaluating hepatic functional reserve in patients with HCC than the aminopyrine breath test, and the amino acid clearance test.[44] Monitoring of ICG elimination is also indicated for the evaluation of liver function in organ donors and recipients in the liver transplantation setting. Several cut-off values for a safe resection have been described in the literature. The ICG-PDR is an important prognostic factor for postoperative recovery and survival,

and has to be higher than 5%/min.[45] Another study reported a safety limit of ICG-R15 of 14% for a major hepatectomy.[44]

However, under certain conditions ICG clearance test results may be misrepresentative of the underlying liver disease, as confirmed by several studies that found no significant correlations between ICG-15 clearance rates and liver histology as well as clinical outcome.[46,47] Mortality has been noted in patients with normal ICG-15 values, and survival has been observed in patients with predicted poor outcome on the basis of preoperative ICG-15 values.[46,48,49] Moreover, factors such as hepatic hemodynamics may influence test outcomes. Variations in hepatic blood flow caused by e.g., intrahepatic shunting or thrombosis will influence ICG clearance rate, rendering the test less predictive.[47] Under pathophysiological circumstances, the transport capacity may be reduced due to downregulation of OATP transporters[50] or by competitive inhibition by excessively present bilirubin.[14] With respect to the former, cytokines such as TNF-α and IL-6, which are released by Kupffer cells in patients with e.g., steatosis and hepatitis, can affect the expression of OATP isoforms and NTCP, thereby affecting ICG uptake by the liver.[15] Consequently, the ICG test may be of limited value during liver diseases in which the parenchyma is compromised, and is improper for cholestatic patients. Another aspect of the ICG clearance test is that it reflects global liver function but does not take into account regional variations that may occur in the liver, particularly under pathological conditions, thereby obscuring a possible functional disadvantage of the segments to be preserved. However, this applies to every clearance test that is performed without an imaging component.

5.2. Galactose elimination capacity (GEC) test

The galactose elimination test determines the metabolic capacity of the liver. Galactose is phosphorylated intracellularly to galactose-1-phosphate by galactokinase. Galactose-1-phosphate is then converted to glucose-1-phosphate by the action of four enzymes in the Leloir pathway.[51,52]

Galactose is administered intravenously, and the GEC is calculated from serial serum samples from 20 to 50 min postinjection, making the test somewhat time-consuming. The GEC has shown prognostic significance in chronic liver disease[53,54], such as fulminant hepatic failure[55], primary biliary cirrhosis[56-58], and chronic active hepatitis.[54,57,59] Abnormal clearance has also been frequently observed in patients with metastatic liver neoplasms.[57] A low GEC-value can predict postoperative complications and death, whereas a high GEC-value is associated with longer survival.[54] As is the case with most liver function tests, alterations in environmental conditions or liver metabolism will affect test outcomes. Galactose is an essential component of membrane glycoproteins, and glycolipids. During liver regeneration, an increased membrane synthesis can lead to an augmented galactose demand.[24] Furthermore, galactose can be converted into glucose, which is used as an energy source during anaerobic respiration, especially during fasting.[24] As a result, altered galactose kinetics during e.g., liver regeneration and fasting[24,60] may provide false positive results with respect to liver function. In addition, GEC only measures total liver function.

6. Molecular nuclear imaging techniques

6.1. ^{99m}Tc-galactosyl serum albumin (GSA) scintigraphy

^{99m}Tc-diethylenetriamine-pentaacetic acid-galactosyl human serum albumin (^{99m}Tc-GSA) is an analogue ligand of asialoglycoprotein (ASGP) that binds to ASGP receptors (ASGP-R) on the hepatocyte cell membrane.[61,62] The ASGP-R consists of 2 subunits, hepatic lectins 1 and 2, and is expressed only on the hepatocyte sinusoidal surface facing the space of Disse.[61-66] ASGPs are taken up by the ASGP-R via receptor-mediated endocytosis. The liver is the only uptake site for ^{99m}Tc-GSA, and it is therefore an ideal agent for receptor-targeted, functional liver scintigraphy. A significant decrease in ASGP-R expression has been observed in patients with chronic liver problems[30], coinciding with the accumulation of ASGPs in the circulation.[67,68]

Planar ^{99m}Tc-GSA scintigraphy has proven valuable for the assessment of liver function in cirrhotic patients, and demonstrated a good relationship with conventional liver function tests such as antithrombin, total and direct bilirubin, PT, ICG clearance[69], Child-Pugh classification, and histology (hepatic activity index (HAI) score).[47,70] A discrepancy between the ICG clearance test and ^{99m}Tc-GSA scintigraphy has been described in 9-20% of the patients, in whom the histological severity of disease was better reflected by ^{99m}Tc-GSA scintigraphy.[71,72] Since bilirubin does not bind to ASGP-R, ^{99m}Tc-GSA scintigraphy is not influenced by hyperbilirubinemia.[73] Also, as there is practically no biliary excretion of ^{99m}Tc-GSA, the radiocompound is perfectly suited for SPECT. On the other hand, the test is not suitable for the evaluation of biliary obstruction as a possible cause of secondary hepatocellular failure, as can be seen in cholangiocarcinoma patients.

In dynamic ^{99m}Tc-GSA scintigraphy, images are obtained after an intravenous bolus of ^{99m}Tc-GSA using a gamma camera positioned over the heart, and liver region. The blood clearance and hepatic uptake are obtained by probing regions of interest (ROIs) in the heart and liver, respectively, for the accumulation of radiolabel in time. For the actual kinetics of ^{99m}Tc-GSA receptor binding, several complex kinetic models have been developed. Although many different parameters can be calculated from different kinetic models, these are often too complex and therefore not widely used in the context of liver surgery.[74] The hepatic uptake ratio of ^{99m}Tc-GSA (LHL15) and the blood clearance ratio (HH15) are the most commonly used parameters in planar dynamic ^{99m}Tc-GSA scintigraphy (Figure 2). The HH15 is calculated by dividing the radioactivity of the heart ROI at 15 minutes after ^{99m}Tc-GSA injection by that at 3 minutes after injection. LHL15 is calculated by dividing the radioactivity of the liver ROI by the radioactivity of the liver plus heart ROIs at 15 minutes after injection.[71,75,76] The modified receptor index (MRI), which represents a quantitative measurement of hepatic function, can subsequently be determined by dividing the LHL15 by the HH15, calculated from the radioactivity of the liver and heart.[71]

Multiple studies have addressed the use of preoperative planar dynamic ^{99m}Tc-GSA scintigraphy for predicting postoperative complications.[61,72,77,78] The preoperative hepatic uptake ratio of ^{99m}Tc-GSA (LHL15) proved a reliable indicator for predicting postoperative complications in patients with HCC and chronic liver disease, showing significantly lower values in patients with major postoperative complications.[47,78] Specific cut-off values for LHL15 (0.900 and 0.875)[72,77,78] have been used to select patients with a high risk for complications. However, these cut-off values were mostly not based on robust risk analysis but rather set arbitrarily. Postoperative liver failure was also observed in patients with a relatively normal liver function (LHL15 >0.875). This can be explained by the fact that LHL15 only measures preoperative total liver function, and not the function of the FRL per se.

^{99m}Tc-GSA scintigraphy can be combined with CT to add anatomical detail to the liver function test. Static ^{99m}Tc-GSA single photon emission computed tomography (SPECT) has the ability to measure segmental liver function, and functional liver volume.[79,80] The outline extraction method is a simple technique to calculate the functional liver volume using a specific cut-off value to automatically outline the liver.[79] Typically, a cut-off value of 35-39% is employed for delineating the liver.[79,81] Although static SPECT has the ability to visualize regional differences in liver function, functional liver volume measured by the outline extraction method, does not take into account the regional functional differences within the delineated liver volume.[74] As an alternative to static SPECT, dynamic SPECT has been employed to measure the uptake dynamics of ^{99m}Tc-GSA in a 3-dimensional manner using a rapidly rotating, multidetector gamma camera.

^{99m}Tc-GSA SPECT provides the opportunity to specifically assess FRL function.[80,82] Preoperative functional volume measured by static ^{99m}Tc-GSA SPECT has proven more suitable for predicting remnant liver function than CT volumetry in a study group with predominantly cirrhotic patients.[79,80] In cirrhotic patients, advanced fibrosis is accompanied by a reduction in functional hepatocytes. The superiority of static ^{99m}Tc-GSA SPECT over CT volumetry can, in this

respect, be explained by the fact that ^{99m}Tc-GSA SPECT measures the functional hepatocyte mass[82], whereas CT volumetry cannot distinguish between functional and non-functional liver tissue. In addition, tumor-induced compression of surrounding liver tissue, bile ducts[79], and/or blood vessels[83] can impact regional liver function, while liver volume is sustained over a longer time period. However, the aforementioned outline extraction method for static SPECT images is based on the assumption that liver function is uniformly distributed in the tissue included within the cut-off value. Especially in tumor-bearing but also compromised livers, function can be distributed heterogeneously.[5,74,84] Therefore, total functional liver volume as measured by static ^{99m}Tc-GSA SPECT does not necessarily correlate with the intrinsic liver function. This potential shortcoming may be circumvented by using dynamic planar ^{99m}Tc-GSA SPECT[76,82] in a 3-dimensional manner (i.e., by using a rapidly rotating, multidetector gamma camera[74,76,82]) with which the intrinsic FRL function can be measured. In addition, dynamic planar ^{99m}Tc-GSA SPECT can be used to predict postoperative complications with a high level of accuracy.[82,85]

6.2. ^{99m}Tc-mebrofenin hepatobiliary scintigraphy

^{99m}Tc-mebrofenin (^{99m}Tc-*N*-(3-bromo-2,4,6-trimethyacetanilide) iminodiacetic acid) is an iminodiacetic acid (IDA) analogue that circulates in albumin-bound form.[44,86,87] Dissociation of mebrofenin from albumin occurs in the space of Disse, after which mebrofenin is taken up by hepatocytes via human OATP1B1, and OATP1B3.[15] Similar to ICG, ^{99m}Tc-mebrofenin undergoes biliary excretion without undergoing biotransformation, and is therefore an ideal tracer for the biliary tract as well.[15,88] The canalicular transporter includes multidrug resistance protein-2.[89] Although ^{99m}Tc-mebrofenin is not metabolized, the transport mechanism resembles the transport of various endogenous, and exogenous substances such as bilirubin, hormones, drugs, and toxins. ^{99m}Tc-mebrofenin hepatobiliary scintigraphy (HBS) therefore measures a physiologically representative function of the liver.

^{99m}Tc-labeled IDA analogues were first used for the diagnosis of multiple biliary diseases.[86,90,91] More recently, the application of ^{99m}Tc-labeled IDA agents have been proposed for the assessment of liver function.[92] Measurement of hepatic uptake function by the clearance rate of the IDA analogue Iodida was first described by Ekman et al.[93] The hepatic uptake of mebrofenin is calculated in a similar manner as that of Iodida. Due to the rapid biliary excretion of mebrofenin, ^{99m}Tc-mebrofenin HBS is primarily used for dynamic rather than static assessment of liver function. After intravenous injection of ^{99m}Tc-mebrofenin, dynamic HBS is performed with a gamma camera[74] as addressed in the previous section on ^{99m}Tc-GSA scintigraphy. To determine global liver function, the hepatic uptake of ^{99m}Tc mebrofenin is determined by assigning a ROI around the liver, the heart (serving as blood pool), and the total field of view, as is illustrated in Figure 3 (top row).

Three different time-activity curves are generated based on these ROIs with which the uptake rate (%/min) can be calculated. Radioactivity values acquired between 150 and 350 seconds post-injection are used to ensure that the calculations are made during a phase of homogenous distribution of the agent in the blood pool, before biliary excretion takes place.[94,95] FRL function can then be calculated by dividing the summed counts (150-350s post injection) within the delineated FRL by the total liver counts within the same time frame, and multiplying this factor with total liver ^{99m}Tc-mebrofenin uptake rate. Finally, total liver ^{99m}Tc-mebrofenin uptake rate (%/min/m^2) can be calculated by dividing the time-activity curve(s) by the patient's body surface area which therefore individualizes the assessment according to patient characteristics.[94,95]

A clinical study compared the ICG clearance test with ^{99m}Tc-mebrofenin HBS in patients undergoing liver resection, and showed a good correlation between the two tests.[95] In a subsequent study, HBS was validated as a tool to measure total liver function as well as FRL function before liver surgery.[94] The latter was validated by comparing preoperative FRL function with actual postoperative remnant liver function immediately after surgery. A strong positive

correlation (r=0.95) was found between FRL function determined preoperatively and the actually measured value 24 hours after resection. Also, 3 months after the resection, there was a strong positive correlation (r=0.81) between liver function assessed by ^{99m}Tc-mebrofenin HBS and the ICG clearance test. A slightly weaker relationship (r=0.61) was found between functional liver regeneration and liver volume increase after 3 months, which may account for some of the discrepancies between volumetric regeneration of the remnant liver and clinical (i.e., functional) outcome after liver resection.[94]

Two clinical studies further validated the utility of ^{99m}Tc-mebrofenin HBS in the preoperative prediction of postoperative liver failure. A study by Dinant et al[87] encompassed 46 patients with and without parenchymal disease. Preoperative measurement of FRL function by planar dynamic ^{99m}Tc-mebrofenin HBS proved more valuable than measurement of FRL volume by CT volumetry for risk assessment of postoperative liver failure and liver failure-related mortality.[87] A safe resection could be performed in patients with an FRL uptake above 2.5 %/min/m^2 body surface area (BSA), with a 3% chance of developing postoperative liver failure and liver failure-related mortality. However, in patients with a FRL uptake below 2.5 %/min/m^2 BSA, the risk of postoperative liver failure increased to 56%. A study by de Graaf et al[6] addressed a population of high-risk patients requiring major hepatic resections (≥3 liver segments), where accurate measurement of FRL function is critical in the assessment of potential resectability. For this population, ROC curve analysis yielded an almost similar FRL function cut-off of 2.7 %/min/m^2 BSA. We now use 2.7 %/min/m^2 BSA as the advised cut-off for FRL function.

Owing to technical advances, new rotating gamma cameras have been developed that enable fast ^{99m}Tc-mebrofenin SPECT, thereby accounting for the rapid hepatic uptake and biliary excretion kinetics of mebrofenin. Dual-head gamma cameras now enable simultaneous data acquisition in the anterior and posterior projections, from which a geometric mean activity can be calculated, thereby reducing the attenuation bias. Additionally, these gamma cameras can be used

in conjunction with CT scanners to combine the functional data from ^{99m}Tc-mebrofenin SPECT with the anatomic information from the CT scan, enabling 3-dimensional measurement of segmental liver function and liver functional volume (Figure 3, bottom row). The FRL can be outlined manually on the low-dose CT scan and linked to the SPECT images. The delineated FRL on contrast-enhanced CT scans can subsequently be used as a constant reference. A recent study, in which FRL function was assessed by ^{99m}Tc-mebrofenin SPECT with low-dose CT, demonstrated that the combination of SPECT with the dynamic uptake data from planar HBS (geometric mean data) allowed complete and accurate prediction of postoperative remnant liver function[5], whereby the SPECT and CT image overlays provided valuable visual information on liver function distribution.

The timing of the SPECT is a challenge when a dynamic tracer such as ^{99m}Tc-mebrofenin is used, which is first taken up by the liver, and subsequently excreted in the bile. The SPECT acquisition is therefore centered around the peak of the hepatic time-activity curve, since the amount of radioactivity within the liver is relatively stable during this phase. In patients with fast hepatic uptake, biliary excretion is already visible during the SPECT phase. Accumulation of radioactivity in the small bile ducts results in voxels with relatively high counts, disturbing calculation of total and regional liver function, and volume. Consequently, the activity within the extrahepatic bile ducts must be masked out, and the intrahepatic bile ducts must be replaced by the average counts of the normal surrounding liver tissue. The outline extraction method can subsequently be used to automatically outline the liver, and calculate total functional liver volume.

Furthermore, ROIs can be drawn around parts of the liver to calculate regional differences in ^{99m}Tc-mebrofenin uptake rate. Segmental liver function, such as that of the FRL, can be measured by dividing the counts within the delineated segment by the total counts within the entire liver. For calculation of the FRL function, this count ratio is multiplied by total liver ^{99m}Tc-

mebrofenin uptake as measured by dynamic HBS. The regional uptake of ^{99m}Tc-mebrofenin can be assessed with small intra- and interobserver variation.[87,94]

Because ^{99m}Tc-mebrofenin HBS has the ability to selectively measure FRL function, it is one of the few liver function tests that can be used to measure the increase in FRL function after PVE. A recent study showed that functional increase of the FRL measured by ^{99m}Tc-mebrofenin HBS exceeds the volumetric increase of the FRL, suggesting that the waiting time until resection may be shorter than indicated by volumetric data.[96]

Liver uptake of IDA agents can be affected by high plasma levels of bilirubin because of the competitive affinity of bilirubin for the respective transporters. Of all IDA analogues, however, ^{99m}Tc-mebrofenin shows the highest hepatic uptake, minimal urinary excretion, and a strong resistance against displacement by high plasma bilirubin concentration.[97] Therefore, ^{99m}Tc-mebrofenin is considered the most suitable IDA analogue for hepatic, and biliary diagnostic procedures. In addition, ^{99m}Tc-mebrofenin uptake can be hindered by hypoalbuminemia, as albumin is the main plasma carrier of mebrofenin. Nevertheless, hypoalbuminemia and hyperbilirubinemia can be a sign of impaired liver function during liver disease, and therefore a decreased uptake of ^{99m}Tc-mebrofenin in patients with hypoalbuminemia can still provide an accurate reflection of liver function under these circumstances.

Discussion

Accurate measurement of liver function before liver resection is crucial in the assessment of hepatic functional reserve and resectability, especially in patients who require major resection and patients with underlying parenchymal disease. For liver surgery, CT volumetry is currently the gold standard method to decide on resectability. Several quantitative liver function tests can complement CT volumetry, and may even replace CT volumetry in the future.

In patients with liver-specific diseases, accurate assessment of liver function is critical for the selection of treatment options. Liver steatosis and steatohepatitis, for example, are associated with an increased risk of liver failure after partial liver resection, especially after neo-adjuvant chemotherapy, or in living donor liver transplantation.[98] When CT volumetry is used as a prognostic tool for surgical outcome, a functional overestimation can be made in patients with steatosis. The accumulation of triacylglycerols in hepatocytes leads to hepatocyte enlargement in combination with steatosis-induced perfusion defects; i.e., phenomena that distort the actual liver function when deduced from CT scans. ICG clearance and ^{99m}Tc-mebrofenin HBS therefore possess the potential to assess hepatic function in steatotic livers because of the combination of impaired parenchymal perfusion, and liver dysfunction.[99]

The same principles apply to cirrhotic livers, where fibrosis is accompanied by a reduction in functional hepatocytes that concurs with the formation of fibrous tissue septa that separate hepatocyte nodules, leading to altered resistance to hepatic blood flow, and portal hypertension.[100,101] The most commonly used liver function tests in cirrhotic patients include hyaluronic acid uptake, the Child-Pugh classification, ^{99m}Tc-GSA scintigraphy, and the ICG test, albeit any liver function test is capable of detecting a reduced number of functional hepatocytes in combination with impaired hepatic blood flow. In any case, liver function tests provide more accurate information on the functional status of the liver than CT volumetry.

Prolonged cholestasis produces hepatocellular injury, and fibrosis. The uptake of ^{99m}Tc-mebrofenin and ICG is impaired under these conditions due to competitive uptake of bilirubin and ICG/mebrofenin by the same cellular transporter systems. Although this impaired uptake is indicative of the uptake function of the liver at that specific time, it does not reflect the function of the liver after surgery once the biliairy obstruction has been resolved. Preoperative assessment of liver function using the ICG clearance test or ^{99m}Tc-mebrofenin HBS therefore requires complete

biliary drainage of at least the FRL in patients with concomitant obstruction of (part of) the biliary tree, as seen in hilar cholangiocarcinomas.

The major disadvantage of most quantitative liver function tests such as the ICG clearance test and the GEC is the fact that they only measure global liver function, and not specifically the function of the FRL. Both ^{99m}Tc-GSA scintigraphy and ^{99m}Tc-mebrofenin HBS have the unique ability to measure FRL function non-invasively. These tests are therefore also suitable for assessing the increase in FRL function after PVE. In addition, they allow for the simultaneous acquisition of morphological (visual), and physiological (functional) information of the liver, especially when SPECT-CT cameras are used. It has been demonstrated in patients that the functional capacity may vary within the liver or even the FRL. With molecular imaging techniques, such regional differences in hepatic function can be detected. In addition, when radiopharmaceutical agents are used that are excreted into the bile, two dynamic phases can be examined, i.e., hepatic uptake of the agent and secretion into the biliary system.

Both preoperative ^{99m}Tc-GSA and ^{99m}Tc-mebrofenin scintigraphy are validated and accurate methods for preoperative assessment of liver function and prediction of postoperative complications.[72,87,95] Both tests are especially suitable for evaluation of liver function in patients with parenchymal liver disease. Unfortunately, the clinical implementation of ^{99m}Tc-GSA scintigraphy has been restricted as to date it has not been approved in Europe and the US, but only in Japan.

In the final analysis, the most suitable liver function test for clinical use is yet to be determined. However, recent studies have provided useful insights into the proper approach towards assessing preoperative liver function. De Graaf et al[24] compared several quantitative liver function tests in a standardized rat model of liver regeneration, including liver volume (CT volumetry), ^{99m}Tc-GSA scintigraphy, ^{99m}Tc-mebrofenin HBS, the ICG clearance test, and the GEC test. It was clearly demonstrated that volumetric assessment of the liver should in any case be

complemented by liver function-specific tests. Due to the functional complexity of the liver, and because each test reflects a different component of liver function, one single liver function test cannot measure liver function comprehensively.[102,103] It is therefore recommended to employ a liver function test that (1) can be combined with imaging (e.g., SPECT) to provide regional functional information, (2) measures a maximum number of components of liver function, and (3) is not restricted in its clinical implementation by regulatory agencies. These selection criteria automatically yield ^{99m}Tc-mebrofenin HBS as the most suitable liver function tests, given that this test (1) can be combined with SPECT, (2) measures the uptake (by two transporters) and excretory function of the liver, and (3) does not face regulatory restrictions such as e.g., ^{99m}Tc-GSA scintigraphy.

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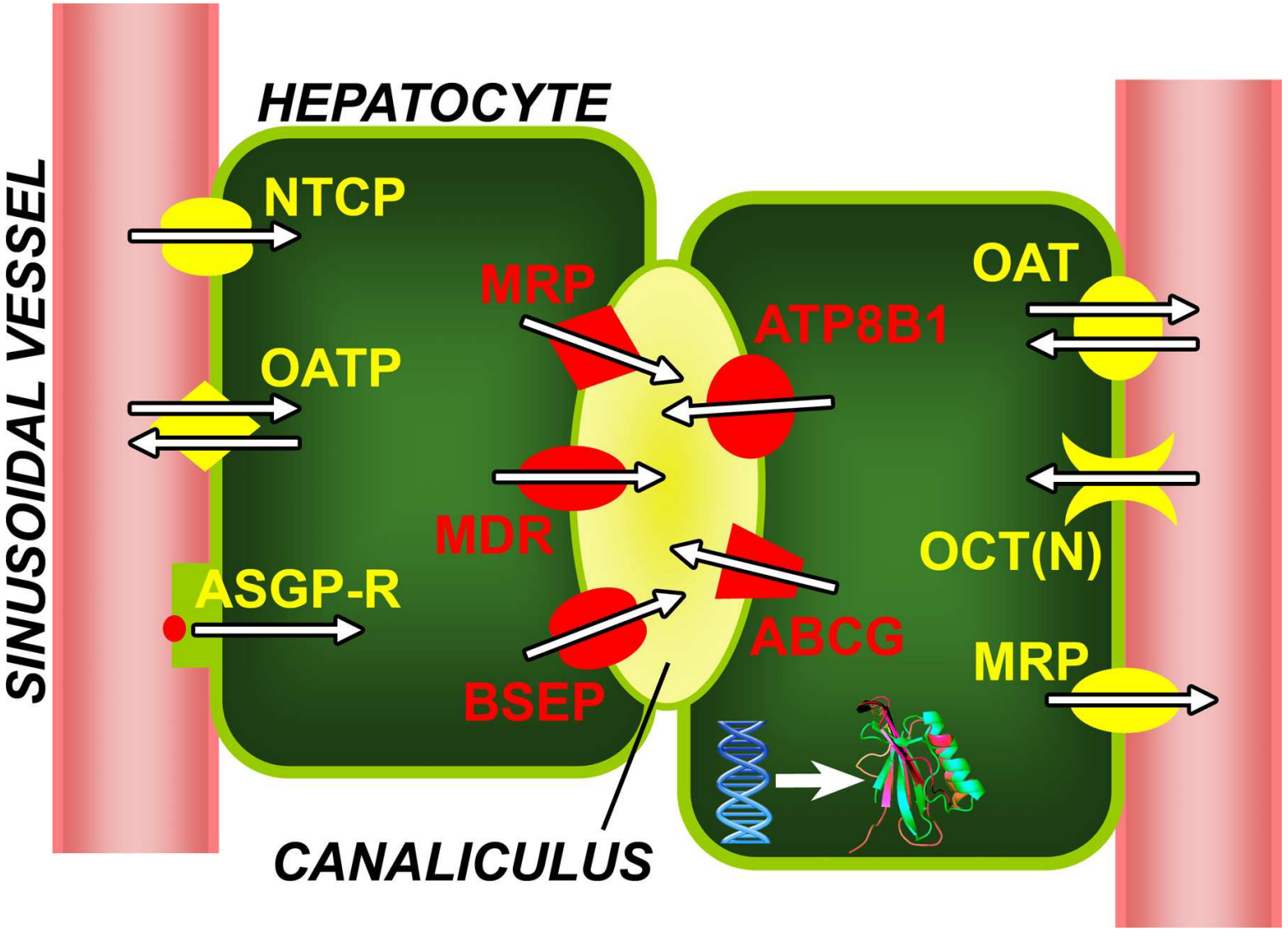
Figure 1. Schematic overview of two prominent features of liver function, basolateral and canalicular transport of compounds and protein synthesis, that are pertinent to this review. The anatomical features are identified and the arrows indicate the direction of transport. Hepatic transporters located at the basolateral side of hepatocytes (yellow structures) are responsible for the transport of compounds from the circulation into the cell (sodium-taurocholate co-transporting polypeptide (NTCP) and the organic cation transporter (OCT) family, comprising the OCT1, OCT2, and OCT3 isoforms), out of the cell (two isoforms of the ATP-binding cassette (ABC) family: multidrug resistance-associated protein (MRP)3 and MRP4), or bidirectionally (the organic anion transporting polypeptide (OATP) family, comprising the OATP1A2, OATP1B1, and OATP1B3 isoforms, and the organic transporter (OAT) family, comprising the OAT2 and OAT3 isoforms). The asialoglycoprotein receptor (ASGP-R) removes asialoglycoproteins from the circulation. Intracellularly transported compounds may undergo biotransformation (e.g., glucoronidation) or be metabolized (these liver function aspects are not shown), after which they may be transported back into the circulation or excreted into bile. All canalicular transporters (red structures) are unidirectional and include several members of the ABC family (MRP2, ATP-binding cassette sub-family B member (ABCG) 1 (also called multidrug resistance (MDR)1), MDR3 P-glycoprotein, and ATP-binding cassette sub-family G members (ABCG) 2, 5, and 8), the bile salt export pump (BSEP), and probable phospholipid-transporting ATPase IC (ATP8B1). The protein synthesis function is indicated by the DNA helix → protein crystal structure.

Figure 2. Planar ^{99m}Tc-GSA scintigraphy. The hepatic uptake ratio (LHL15) and blood disappearance ratio (HH15) is calculated from the ^{99m}Tc-GSA time-activity curves from the heart (grey) and the liver (black) (left panel). The HH15 is calculated by dividing the radioactivity of the heart ROI at 15 minutes after ^{99m}Tc-GSA injection by that at 3 minutes after injection. LHL15 is calculated by dividing the radioactivity of the liver ROI by the radioactivity of the liver plus heart ROIs at 15 minutes after injection.[75]The subscript values designate time, the capitalized letters indicate the organ according to the legend. The blood disappearance constant (K_L) is calculated from the liver uptake curve using the disappearance halftime ($T_{1/2}$) (right panel). Images adapted from [74].

Figure 3. ^{99m}Tc-mebrofenin hepatobiliary scintigraphy (HBS, top row) and ^{99m}Tc-mebrofenin SPECT (bottom row). The upper left panel shows superimposed images of dynamic planar HBS scans from 150-350 sec after intravenous injection of ^{99m}Tc-mebrofenin, which ensures that hepatic uptake calculations are performed during a phase of homogenous distribution of ^{99m}Tc-mebrofenin**.** A region of interest is drawn around the entire liver (red line), the mediastinum (blood pool, yellow line), the total field of view (not shown) and the future remnant liver (FRL, green line). The respective, color-coded time-activity curves are depicted in the upper right panel. The time-activity curves of the liver (segments) are typically corrected for background, i.e., the blood pool time-activity curve, by subtraction. The uptake of ^{99m}Tc-mebrofenin (D) by the liver is calculated as an increase in blood pool-corrected ^{99m}Tc-mebrofenin uptake (y) over a predefined time interval (x), usually 200 seconds.
The bottom left and right panels depict an example of ^{99m}Tc-mebrofenin SPECT with a matching CT scan. An inhomogeneous distribution of ^{99m}Tc-mebrofenin is seen in liver segments 7 and 8 (arrows) due to regional cholestasis in a patient with hilar cholangiocarcinoma. The matching CT scan shows dilated bile ducts (arrowheads) in the same liver segments.


SINUSOIDAL VESSEL
HEPATOCYTE
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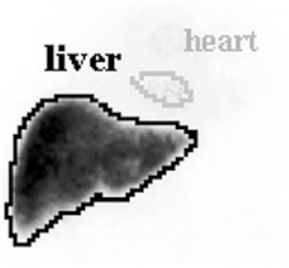
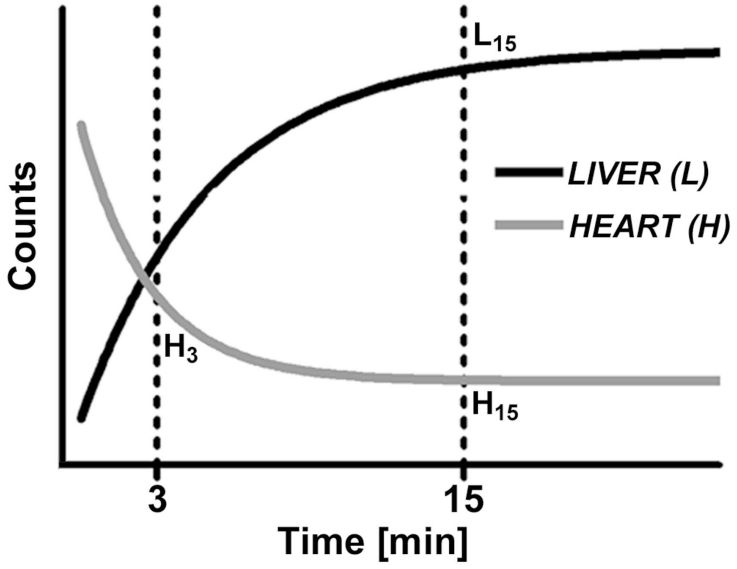
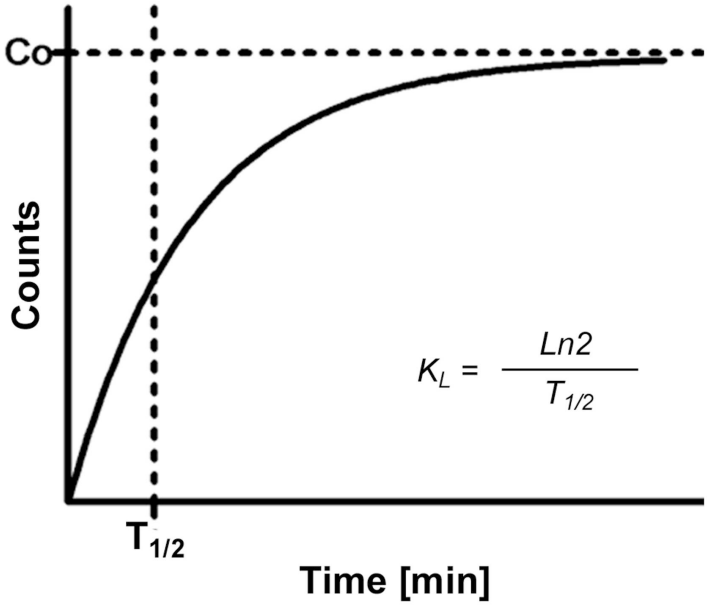


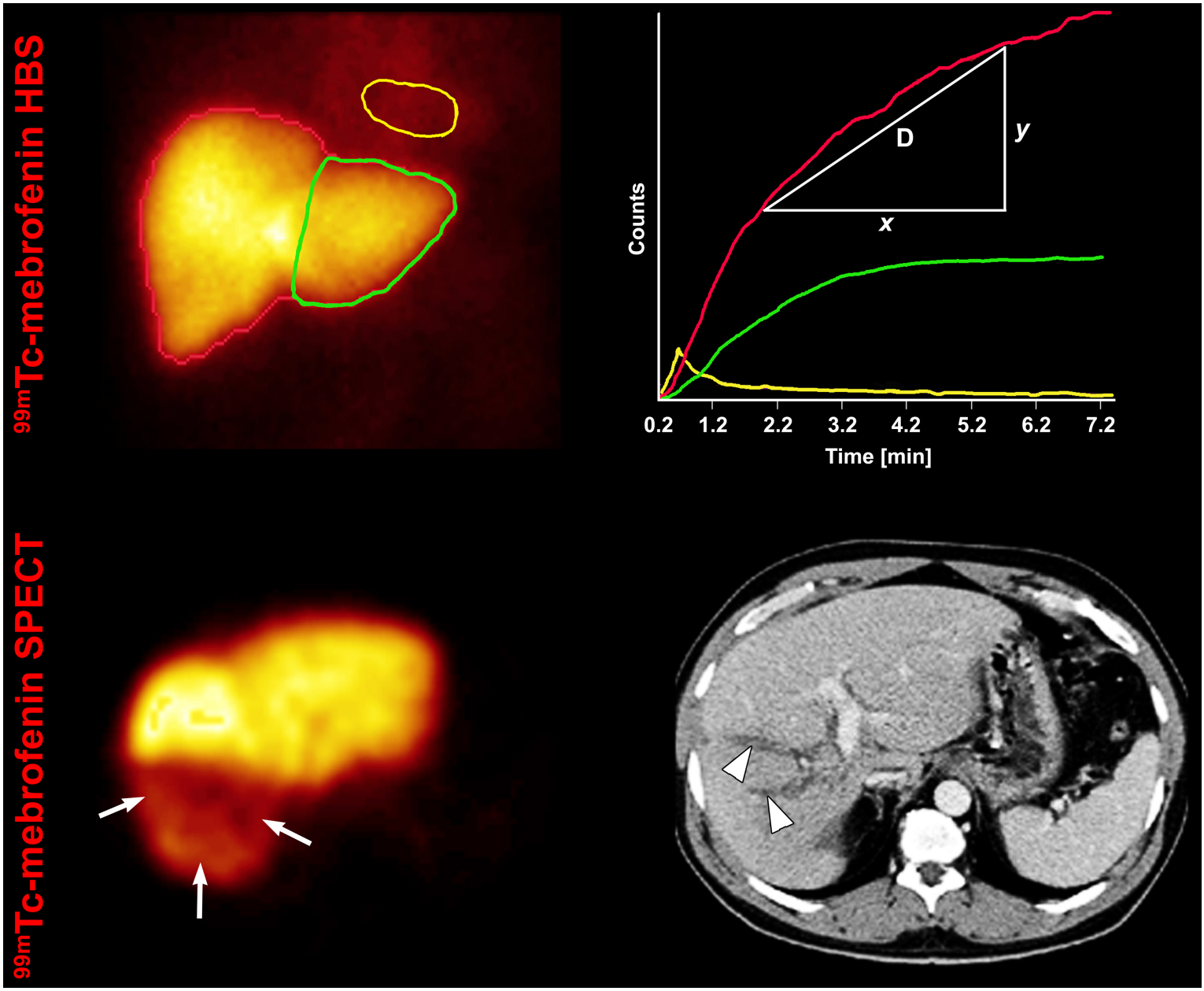
$$LHL15 = \frac{L_{15}}{L_{15}+H_{15}}$$

$$HH15 = \frac{H_{15}}{H_3}$$





$$K_L = \frac{Ln2}{T_{1/2}}$$


99mTc-mebrofenin HBS
Counts
D
y
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1.2
2.2
3.2
4.2
5.2
6.2
7.2
Time [min]
99mTc-mebrofenin SPECT